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***Yersinia enterocolitica* specific infection by bacteriophages TG1 and ϕ R1-RT is
dependent on temperature regulated expression of the phage host receptor
OmpF**

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ABSTRACT

Bacteriophages present huge potential both as a resource for developing novel tools for bacterial diagnostics and for use in phage therapy. This is also valid for bacteriophages specific for *Yersinia enterocolitica*. To increase our knowledge on *Y. enterocolitica* –specific phages we characterized two novel yersiniophages. The genomes of the bacteriophages vB_YenM_TG1 (TG1) and vB_YenM_φR1-RT (φR1-RT), isolated from pig manure in Canada and from sewage in Finland, consist of linear double-stranded DNA of 162,101 and 168,809 bp respectively. Their genomes encode 262 putative coding sequences and 4 tRNAs genes, and share 91% overall nucleotide identity. Based on phylogenetic analyses of their whole genome sequences and large terminase subunit protein sequences, a genus named *Tglvirus* within the family *Myoviridae* is proposed with TG1 and φR1-RT as member species. These bacteriophages exhibit a host range restricted to *Y. enterocolitica*, and display lytic activity against the epidemiologically significant serotypes O:3, O:5,27, and O:9 at and below 25°C. Adsorption analyses of LPS and OmpF mutants demonstrate that these phages use both the LPS inner core heptosyl residues and the outer membrane protein OmpF as phage receptors. Based on RNA-sequencing and quantitative proteomics we also demonstrate the temperature dependent infection is due to strong repression of OmpF at 37°C. In addition, φR1-RT was shown to be able to enter into a pseudolysogenic state. All together, this work provides further insight into phage-host cell interactions by highlighting the importance of understanding underlying factors which may affect the abundance of phage host receptors on the cell surface.

IMPORTANCE

54 Only a small number of bacteriophages infecting *Y. enterocolitica*, the predominant
55 causative agent of yersiniosis, have been previously described. Here, two newly
56 isolated *Y. enterocolitica* phages were studied in detail with the aim of elucidating the
57 host cell receptors required for infection. Our research further expands the repertoire
58 of phages available for consideration as potential antimicrobial agents or as diagnostic
59 tools for this important bacterial pathogen.

INTRODUCTION

Yersinia enterocolitica, a facultative anaerobic, Gram-negative, non-sporulating, short bacillus isolated frequently from soil, water, animals, and foods, is an important zoonotic pathogen leading to human and animal enteric infection (1). The main animal reservoir for *Y. enterocolitica* is pigs, and pork derived products are thought to be the main source of human infections in addition to drinking of contaminated water and blood-transfusions (1, 2). Symptoms of yersiniosis may include diarrhea, terminal ileitis, mesenteric lymphadenitis, and septicemia (3). Among the species within the genus *Yersinia*, *Y. enterocolitica* is highly heterogeneous and is grouped into six phylogroups (4). The widely used bioserotype groups form the basis of the phylogroups such that phylogroup 1 contains the biotype 1A strains, phylogroup 2 the highly pathogenic biotype 1B strains, phylogroup 3 the bioserotype 4/O:3 strains, phylogroup 4 bioserotype 3/O:9 strains, phylogroup 5 bioserotype 2/O:5,27 strains and phylogroup 6 the serotype O:2,3 strains rarely isolated from hares (4–7). *Y. enterocolitica* is also represented by over 60 serotypes that are determined by the variability of O-antigens present in the outer cell membrane (8, 9). The predominant pathogenic strains associated with yersiniosis belong to bioserotypes 1B/O:8, 2/O:5,27, 2/O:9, 3/O:3, and 4/O:3, with the last being the most common in Europe, Japan, Canada, and the United States (1, 2). From 2010–2012, 98% of all reported yersiniosis infections worldwide were acquired in Europe, and most (97%) were caused by *Y. enterocolitica*, with the remainder caused by *Y. pseudotuberculosis* (10). In 2015, the most commonly reported *Y. enterocolitica* serotype in the European Union was O:3 (89%), followed by serotypes O:9 (7%), O:5,27 (2%) and O:8 (2%) (10).

Although several bacteriophages infecting *Y. enterocolitica* have been described, few have been studied in detail providing reliable information on morphology, host range, and or receptor specificity. To date, bacteriophages ϕ YeO3-12(11–13) and vB_YenP_AP5 (14) with specificity for *Y. enterocolitica* O:3, phage PY54 exhibiting a host range restricted to *Y. enterocolitica* O:5 and O:5,27 (15), *Yersinia* phage ϕ R1-37 with a broad host range within the species *Y. enterocolitica* (16, 17) and *Yersinia* phage PY-100 (18) exhibiting a broader host range restricted to the genus *Yersinia*, have been described. These bacteriophages use different parts of the *Y. enterocolitica* lipopolysaccharide (LPS) as receptors (19). Analysis of the host-range combined with genetic and structural data have shown that the receptor for ϕ R1-37 is the *Y. enterocolitica* O:3 LPS outer core (OC) hexasaccharide (16). The host receptor for phages ϕ YeO3-12 and vB_YenP_AP5 has been determined to be the LPS O-antigen of serotype O:3 consisting of the sugar 6-deoxy-L-altropyranose (12, 14, 20). Given the interest in bacteriophages because of their potential use as therapeutic, diagnostic, and bio-control agents, the aim of this study was to characterize two newly isolated bacteriophages that are active against several epidemiologically significant *Y. enterocolitica* serotypes. In this study, the genome characterization, morphology, host range, host cell receptor specificity, and taxonomic position of the myovirus phages vB_YenM_TG1 (hereafter TG1) and vB_YenM_ ϕ R1-RT (hereafter ϕ R1-RT) are described.

MATERIALS AND METHODS

Bacterial strains, phage isolation, and growth conditions. Bacterial strains, bacteriophages and plasmids are listed in **Table 1**. Bacteriophage ϕ R1-RT was isolated from the incoming sewage of the Turku (Finland) city sewage treatment

plant, as described for other viruses (19) whereas bacteriophage TG1 was isolated from pig manure collected from a rural farm in Ontario, Canada as described previously for the isolation of *Y. enterocolitica* phages for phagetyping (21). For DNA extraction and morphological studies, ϕ R1-RT was propagated on *Y. enterocolitica* strain YeO3-R1 (22) and TG1 on *Y. enterocolitica* strain YeO3-c (23).

Electron Microscopy. The preparation of the phage particles for transmission electron microscopy (TEM) was done as described (17, 24). Details are presented in Supplementary Materials and Methods.

Host Range. The lytic activity of ϕ R1-RT and TG1 was tested on 109 and 160 strains (Table S1), respectively, belonging to 13 *Yersinia* species, as determined by standard spot tests (24). Briefly, 10 μ l from a phage suspension containing approximately 10^8 PFU were spotted in the middle of a lawn of bacteria incubating for 18-24 h. Each strain was tested three times at 25°C and at 37 °C. Bacterial strains were considered sensitive to the phage if the degree of lysis was observed as a complete clearing, clearing throughout but with a faint hazy background, substantial turbidity throughout the cleared zone, or a few individual plaques (24). Bacterial strains were considered resistant if there was no effect of the phage on bacterial growth.

Genome sequencing and assembly. Details of the determination of the genomic sequences of phages ϕ R1-RT and TG1 as well as the draft genomes of *Y. enterocolitica* strains YeO3- ϕ R1-RT-R2, -R7 and -R9 are presented in Supplementary Materials and Methods.

Bioinformatics. Detailed description of the bioinformatics tools used is given in Supplementary Materials and Methods.

Complementation of the *Y. enterocolitica* O:3 OmpF mutant. The full ORF of *ompF* gene plus the upstream promoter region of YeO3-c was cloned as a 2 kb PCR fragment that was amplified with Phusion DNA polymerase using primer pair OmpC-F2 and OmpC-R2 (**Table S2**) into plasmids pTM100 and pSW25T to obtain plasmids pTM100_OmpF and pSW25T_OmpF, respectively (**Table 1**). Briefly, the PCR fragments were digested with MfeI and ligated with EcoRI digested, SAP-treated pTM100 or pSW25T. The constructed plasmids were mobilized to the OmpF mutant strain YeO3-c-R1-Cat17 by diparental conjugation as described earlier (25).

Phage adsorption assay. To identify the phage cell host receptors, a variety of *Y. enterocolitica* O:3 mutants (**Table 1**) were utilized in phage adsorption experiments. Approximately 5×10^3 PFU of phage ϕ R1-RT or phage TG1 in 100 μ l was mixed with a 400- μ l sample of bacteria ($A_{600} \sim 1.2$). The suspension was incubated at RT for 5 min and centrifuged at 16,000 g for 3 min, and the phage titer remaining in the supernatant, *i.e.*, the residual PFU percentage, was determined. LB was used as a non-adsorbing control in each assay, and the phage titer in the control supernatant was set to 100%. Each assay was performed in duplicate and repeated at least three times.

Total RNA extraction and RNA sequencing. Detailed description of the methods is presented in Supplementary information Materials and Methods. The RNA sequence data has been deposited to Gene Expression Omnibus (accession number GSE66516).

Quantitative proteomics. Detailed description of the methods is presented in Supplementary information Materials and Methods.

Transduction assay. *Y. enterocolitica* O:3 strain YeO3-*hfq*::Km with *hfq* gene knocked-out with a kanamycin resistance cassette (**Table 1**) was used as a donor and transducing particles were produced by infecting this strain with phage ϕ R1-RT using the soft agar overlay method. Following overnight incubation, phages were eluted from the soft agar using SM buffer. The transducing lysates were centrifuged and treated with chloroform to prevent contamination with the donor strain. The titer of the obtained transducing stock was 6.62×10^9 PFU/mL. The *Y. enterocolitica* strain 6471/76 was used as the recipient. For the transduction of the recipient strain, 10 mL aliquots of log-phase bacterial cultures containing 10^9 CFU/mL cells were mixed with 100 μ l of 10^{-2} diluted transducing phage stock resulting at MOI of 0.006. After 15 min the bacterial cells were centrifuged and washed with LB and centrifuging them down removed the unabsorbed phages. The final cell pellet was resuspended in 100 μ l LB, and the cells were allowed to recover during 30 min incubation with vigorous shaking. Subsequently, the bacterial cultures were plated on urea agar plates (0.1% peptone, 0.1% glucose, 0.5% NaCl, 0.2% KH_2PO_4 , 0.00012% phenol red, 2% urea, 1.5% agar) supplemented with kanamycin (200 μ g/mL) and incubated for 48h. The kanamycin resistant and urease negative colonies were considered as transduced. The transducing stock was also plated to ensure no contamination with donor strain.

Growth curves. Overnight bacterial cultures were diluted 1:10 in fresh LB medium and 180 μ l aliquots were distributed into honeycomb plate wells (Growth Curves Ab Ltd) where they were mixed with 20 μ l aliquots of different ϕ R1-RT phage stock

dilutions ($10^0 - 10^{-4}$). A negative control was obtained by mixing 20 μ l of phage stock with 180 μ l of medium, whereas positive control consisted of 180 μ l of bacterial culture and 20 μ l of medium. The growth experiments were carried out at 4°C, 10°C, 16°C, 22°C, and 37°C using the Bioscreen C incubator (Growth Curves Ab Ltd) with continuous shaking. The OD₆₀₀ of the cultures was measured at selected time intervals. The averages were calculated from values obtained for the bacteria grown in 5 parallel wells.

Phage resistant mutant isolation. A culture of wild type *Y. enterocolitica* strain 6471/76 was used to flood LB agar plates (LA). After the excess fluid was removed the plates were allowed to dry before two 100 μ l aliquots of the ϕ R1-RT stock were pipetted on the lawn of cells. The plates were incubated at 22°C and inspected daily for phage resistant colonies growing within the lysis zones. After three days several colonies appeared and among them three confirmed phage resistant derivatives were isolated. The strains were named YeO3- ϕ R1-RT-R2, YeO3- ϕ R1-RT-R7, and YeO3- ϕ R1-RT-R9.

CatMu-library screening. The *CatMu*-transposon insertion library in *Y. enterocolitica* strain YeO3-R1 has been described elsewhere (26, 27). In the present work, a library representing 16,000 independent insertion mutants was screened. The library was grown in LA supplemented with 100 μ g/ml chloramphenicol (LA-CIm) until OD₆₀₀ = ~0.5. Phage ϕ R1-RT was added to 1 mL of the library culture at MOI ~10, fresh LB added to 5 mL and the culture was incubated at 22°C for 2h during which time all phage-sensitive bacteria were expected to be infected and lysed. The surviving bacteria were pelleted by centrifugation, washed twice with 1 mL LB to

remove remaining phages and after resuspending into 100 µl of LB plated on four LA-Clm plates that were incubated at 22°C. The Clm^R colonies were re-streaked on LA-Clm plates for further study.

Arbitrary PCR. Detailed description of the method is presented in Supplementary information Materials and Methods.

Cloning, expression and purification of the phage long tail fiber host receptor binding protein. The phage TG1 distal long tail fiber (LTF) protein Gp37 was co-expressed with phage encoded chaperones Gp57A and Gp38 to synthesize the native form of the putative receptor binding protein (RBP) as described previously for the LTF of phage T4 (28). The Gp37 encoding gene was first cloned into the multiple cloning site (MCS) 1 of pCDF Duet-1 (conferring streptomycin resistance), producing pCDF Duet-1 Gp37. Then, the Gp38 encoding gene was cloned into the MCS 2 of pCDF Duet-1 Gp37, yielding pCDF Duet-1 Gp37-Gp38. Plasmid pET21a(+) conferring ampicillin resistance was used to clone the chaperone Gp57A encoding gene yielding plasmid pET21a(+) Gp57A. The plasmid constructs carry under the control of promoter T7, high level inducible gene expression with a His₆ fusion tag at the N-terminus for purification by chelating affinity chromatography (**Fig. S1**). The genes encoding Gp38 and Gp57A however, were expressed without a purification tag. PCR, restriction analysis, and DNA sequencing were used to verify the structure of the plasmids. For expression, *E. coli* BL21 StarTM (DE3) PLysS cells (Invitrogen) were transformed with pCDF-Duet-1 Gp37 or pCDF-Duet-1 Gp37-Gp38 and the same plasmids were also co-transformed with pET21a(+) Gp57A. Plasmid bearing *E. coli* were grown aerobically at 37°C to an OD₆₀₀ = ~0.6 with shaking at 200 rpm in

234 250 mL of 2xYT media (16 g/L tryptone, 10 g/L yeast extract, 5.0 g/L NaCl, 0.22 μ m
235 filter sterilized, pH 6.5-7.5) supplemented with 50 μ g/mL of ampicillin and or 50
236 μ g/mL streptomycin as required. Protein expression was induced by the addition of 1
237 mM isopropyl-d-1-thiogalactopyranoside (IPTG) (Sigma-Aldrich, USA) incubating
238 for 24h at 30°C with shaking at 200 rpm. Cells were harvested by centrifugation at
239 10,000 g for 15 min at 4°C and the pellets were resuspended in 25 mL of buffer A (50
240 mM sodium phosphate, 300 mM NaCl, 10mM imidazole, pH 8.0) supplemented with
241 a protease inhibitor cocktail (Roche). Cells were disrupted by 10 rounds of 15 s of
242 sonication using a Virsonic Digital 475 ultrasonicator (VirTis, NY, USA) alternating
243 with incubation on ice. Insoluble debris was removed by centrifugation at 18,000 g
244 for 30 min at 4°C and the soluble fraction was filtered through a 0.22 μ m pore size
245 filter (EMD Millipore, USA). The protein was purified by immobilized metal ion
246 affinity chromatography using a nickel-nitrilotriacetic acid (Ni-NTA) agarose column
247 (Novex, Invitrogen) according to the manufacturer's protocol. Captured proteins were
248 eluted from the column using buffer B (50 mM sodium phosphate, 300 mM NaCl,
249 500mM imidazole, pH 8.0) and concentrated using Amicon-Pro centrifuge filters
250 (Millipore) with a 10,000 Da molecular mass exclusion limit incorporating three
251 washes with 10 mM Tris-HCl of pH 8.5. Protein concentration was estimated by
252 measuring sample absorbance at 280 and 260 nm using a Nanodrop 2000 UV-vis
253 Spectrophotometer (Thermo Scientific, USA) and Qubit® Protein Assay Kit using a
254 Qubit® 1.0 fluorometer (Life Technologies) as per the manufacturer instructions.
255 Protein analysis was performed by Sodium dodecyl sulfate-polyacrylamide gel
256 electrophoresis (SDS-PAGE) (29) using Mini-Protean®TGX Stain-Free Precast Gels
257 (Bio-Rad Laboratories, USA) and Coomassie blue staining. Precision Plus Protein™
258 Unstained Standard (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used as a

size marker for the molecular analysis of proteins. Analysis of protein bands and molecular weight (MW) estimates was performed using a Molecular Imager® Gel Doc™ XR+ System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and Quantity One® software (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Accurate MW determinations and Peptide mass fingerprinting analysis was performed via mass spectrometry (MS) at the Mass Spectrometry Facility, Advanced Analysis Centre of the University of Guelph (Ontario, Canada).

Cell decoration with bacteriophage host recognition binding proteins. Confocal laser immunofluorescent microscopy was used to visualize the binding of the phage TG1 LTF protein Gp37 to *Y. enterocolitica* following methodology described by others (30). *Yersinia* strains grown in TSB at 25°C or 37°C for 24 hours were resuspended in wash buffer (50 mM Tris-HCl, pH 7.5) and 10 µl were spotted onto clean glass slides. After air-drying, the cells were fixed in a solution of 5 % gluteraldehyde for 10 min. and blocked with blocking buffer (5% BSA in 50 mM Tris-HCl buffer, pH 7.5) for 10 min. The slides were then incubated for 1 hour in a solution containing of 10 µg/mL of phage TG1 Gp37 (prepared in blocking buffer) followed by washing three times for 5 minutes in wash buffer. The slides were then incubated for 1 hour in anti-His₆ tag (HIS.H8) mouse monoclonal antibody solution prepared in blocking buffer (1:1000 dilution) and washed three times for 5 minutes with wash buffer. In a dark room, the slides were then incubated for 1 hour in goat anti-mouse IgG DyLight 488 polyclonal antibody solution (1:500) prepared in blocking buffer and washed three times for 5 minutes with wash buffer. The slides were air dried prior to analysis. Cells were imaged using an upright Leica DM 6000B confocal laser microscope connected to a Leica TCS SP5 system. Images were

collected digitally using Leica LAS AF Imaging Software and processed using ImageJ (31). To verify the specificity of the fluorescent signal, control samples were immunolabelled as above, with the omission of incubation with the primary antibody. All antibodies were acquired from Pierce Scientific, USA.

Genome sequences. The complete genome sequences of *Yersinia* phage vB_YenM_TG1 and vB_YenM_φR1-RT were deposited in the NCBI nucleotide database (GenBank) under the accession numbers KP202158 and HE956709, respectively. The RNA sequence data has been deposited to Gene Expression Omnibus (Acc. no GSE66516).

RESULTS

Phage Morphology. Phages φR1-RT and TG1 were negatively stained and examined by TEM. Both phages exhibit a prolate head with apparent icosahedral symmetry and a tubular contractile and rigid tail showing transverse striations (**Fig. 1**). The average dimension for the φR1-RT head is 82 ± 4 nm short edge-to-edge, 101 ± 5 nm vertex-to-vertex and the tail including the baseplate is on average 130 ± 7 nm long. The average dimension for the TG1 head is 91 ± 2 nm short edge-to-edge, 115 ± 6 nm vertex-to-vertex and the tail including the baseplate is on average 129 ± 1 nm long. Collectively, these morphological features indicate that these phages belong to the *Myoviridae* family.

Host specificity. The host range of phages TG1 and φR1-RT were determined by testing their lytic activity on 160 and 109 strains, respectively, belonging to thirteen *Yersinia* species, revealing virulence for *Y. enterocolitica* strains of serotypes O:1,

O:2, O:3, O:5, O:6, O:5,27, O:7,8, O:9 and some strains of serotype O:6,30 and O:6,31 while strains from other *Y. enterocolitica* serotypes and species within the genus *Yersinia* were resistant to phage infection (**Table 2**). TG1 and ϕ R1-RT lysed their host when grown at 25°C but not at 37°C. Additionally, TG1 was unable to infect strains belonging to other 20 other genera (**Table S3**) demonstrating the phages' host range is restricted to *Y. enterocolitica*.

General features of the phage genomes. The genome of phage ϕ R1-RT is 168,809 bp long with a GC content of 34.5%. The genome encodes 262 ORFs, of which 217 genes are encoded on the reverse strand (as displayed on the genetic map) and 45 genes on the forward strand (**Fig. S2**), with sizes ranging from 117 bp (product of 38 amino acids) to 3,738 bp (product of 1245 amino acids). The genome of TG1 is smaller than that of ϕ R1-RT at 162,101 bp in length but with a similarly low GC content of 34.6%. TG1 also encodes 262 ORFs of which 223 genes are transcribed on the reverse strand (as displayed on the genetic map) and thirty-nine genes on the forward strand (**Fig. S3**), with sizes ranging from 114 bp (product of 37 amino acids) to 3,099 bp (product of 1032 amino acids). The GC content of these phages is significantly lower than that associated with the host with a GC content ranging from 47.1 ± 0.2 (32) to 48.5 ± 1.5 % (33). The genomes encode additionally four identical tRNA genes (Gly_{GGA}, Trp_{TGG}, Arg_{AGA}, Met_{ATG}) identified using tRNAScan (34) and ARAGORN (35). Constitutively low GC phage genomes are often supplemented with tRNA genes that, once expressed, enhance translation efficiency when infecting high GC content hosts (36). At the DNA level the TG1 genome shows 98% identity with a query coverage of 93%, for an overall DNA sequence identity of 91% with ϕ R1-RT. All ORFs were screened using the BLASTP and PSI-BLAST algorithms (37, 38).

Based on protein homology, putative functions could be assigned to 121 (46%) gene products of phage TG1 and 115 gene products (44 %) of phage ϕ R1-RT. Most of the identified homologs are conserved among T4-like phages and are either structural, or involved in DNA replication, recombination, repair, or packaging. Thus, the phage T4 gene nomenclature was used to name these genes (**Table S4**).

DNA replication, recombination, and repair. Numerous genes were identified within the phage ϕ R1-RT and TG1 genomes that play a direct role in DNA replication, recombination, and repair. Among the genes directly involved in DNA replication are a DNA polymerase, a DNA ligase (Gp30), and three proteins with helicase activity. The closest homologs to the phage TG1 and ϕ R1-RT polymerases are found in *Edwardsiella* phage PEi20 [BAQ22701.1] and *Enterobacteria* phage RB69 [NP_861746.1]; all members of the *Myoviridae*. Among the helicases, Gp41 (or Dda) and UvsW homologs are involved in the reorganization of stalled DNA replication forks (39). Other putative proteins identified include homologs to the DNA polymerase sliding clamp loader complex Gp44/Gp62, sliding clamp accessory protein Gp45, single-stranded DNA binding protein Gp32, DNA helicase loader Gp59, and Gp61. In phage T4, the latter is a primase that interacts with helicase Gp41 to form a helicase-primase complex (or primosome). The primosome together with the DNA helicase loader Gp59, unwinds the DNA template and primes DNA synthesis on the discontinuous strand. Among the proteins involved in recombination are type II topoisomerases Gp60 and Gp52, the recombination-related endonuclease pair Gp46/Gp47, the Rec-A like recombination protein UvsX, and a single stranded DNA binding protein, UvsY (40). Lastly, among the proteins involved in repair, a DenV homolog and several RNA ligases were identified. DenV is an N-glycosylase

UV repair enzyme that excises pyrimidine dimers; the major UV-lesions of DNA, while RNA ligases seal breaks in RNA and may also counteract host defense of cleavage of specific tRNA molecules (41).

Nucleotide metabolism. Class I ribonucleotide reductases are responsible for the inter-conversion of ribo- to deoxyribonucleotides and are represented by NrdA-B or NrdE-F which require oxygen for activity, class II containing NrdJ, and the oxygen sensitive class III represented by NrdG-H (42). In TG1 and ϕ R1-RT, genes coding for the aerobic ribonucleotide reductase complex subunits NrdA, NrdB, and NrdH were identified. Additionally, NrdC genes were also located. Other genes identified that are involved in nucleotide metabolism include: thymidylate synthase (*Td*), thymidine kinase (*Tk*), dNMP kinase, dCMP deaminase (*Cd*), dihydrofolate reductase (*Fdr*), dCTPase-dUTPase, and the exo-deoxyribonuclease *DexA* and endo-deoxyribonuclease *DenA*. A combination of at least some of these genes is required to supplement the intracellular pool of nucleotides for phage DNA and RNA synthesis (41).

Transcription. Based on the genome maps presented (**Fig. S2 and S3**), phage TG1 and ϕ R1-RT present a similar gene arrangement. A search for promoters based on sequence similarity to the host consensus *s*70 promoter TTGACA(N15-18)TATAAT with a 2 bp mismatch, identified 22 probable host promoters in the phage TG1 genome and 24 probable host promoters in the ϕ R1-RT genome which probably function in early transcription (**Tables S5 and S6**). Additionally, 15 of the putative host promoters are located in the same relative genomic positions within each phage genome. The genomic layout however, makes it clear that there must be additional

promoters functioning to direct the transition from host to viral metabolism. A search for phage-specific promoters using PHIRE (43) and by analysis of sequences of 100 bp in length upstream of each ORF and submitting them to MEME (44), did not yield additional promoters that could be annotated with confidence. A search for putative rho-independent transcription terminators using ARNold (45, 46) yielded 21 putative terminators in the phage TG1 genome (**Table S7**) and 24 in the phage ϕ R1-RT genome (**Table S8**). Nevertheless, the presence phage T4 homologs involved in the transcription of late genes: RegA, Gp33, and the sigma factor for late transcription Gp55, suggest that the mechanism for controlling late transcription is similarly complex (41). Likewise, the presence of repressor and translational regulatory protein homologs involved in middle and late transcription including: RegB, DsbA, Alc, MotA, and AsiA, lend further support to this suggestion.

Morphogenesis. The putative structural proteins of TG1 and ϕ R1-RT are homologous to existing phage proteins of the T4 supergroup of viruses (**Table S4**). Among the putative phage structural genes, the phage head is likely composed of the major capsid protein Gp23 and the phage capsid vertex protein Gp24. The prohead precursor and scaffolding proteins Gp68, and Gp67 as well as internal head proteins ipIII and ipII were also identified. Lastly the head portal vertex protein Gp20 that is connected to the neck and through which DNA enters during packaging and exits during infection was also identified. The whiskers and neck are composed of fibrillin (*wac*) and the head completion proteins Gp13 and Gp14. The tail proteins include the tail sheath terminator Gp3, the tail completion protein Gp15, the tail sheath subunit Gp18, and the tail tube subunit Gp19. Proteins that form the baseplate wedge subunits and tail pins that then go on to associate with the central hub to form the viral

baseplate include: Gp5, Gp6, Gp7, Gp8, Gp9, Gp10, Gp11, Gp25, Gp27, Gp28, and Gp53. Among these, Gp5 (*ORF150*) contains a predicted bacteriophage T4-like lysozyme domain (cd00735) or Phage lysozyme domain (pfam00959), which aids penetration through the peptidoglycan layer during the initial infection process. In phage T4, Gp8 and Gp9 connect the long tail fibers of the virus to the baseplate and trigger tail contraction after viral attachment to a host cell, while Gp11 connects the short tail fiber protein Gp12 (*ORF159*) to the baseplate (47). The baseplate wedge subunit Gp25, forms a structural component of the outer wedge of the baseplate that has lysozyme activity, evident by the presence of conserved Gene 25-like lysozyme domain (pfam04965). Based on homology and gene synteny the proteins forming the long tail fibers in TG1 and ϕ R1-RT are composed of the tail fiber proximal subunit Gp34, the tail fiber connector or hinge protein Gp35, the proximal tail fiber protein Gp36, and the distal tail fiber protein Gp37 (47). A variety of chaperones or assembly catalysts involved in morphogenesis were also discovered. Head formation chaperones include the capsid vertex assembly chaperone, the prohead assembly proteins Gp21 and Gp22, as well as the head assembly chaperone protein Gp31. Chaperones involved in tail formation include the baseplate hub assembly proteins Gp26 and Gp51. Chaperones for tail fiber assembly include gp57A, gp57B, and Gp38.

Host cell recognition elements. In phage T4, phage tail associated receptor-binding proteins (RBPs) Gp37 and Gp12 are necessary for host cell recognition, attachment, and initiation of infection. In the phage TG1 and ϕ R1-RT genomes *ORF250* codes for a putative RBP protein of 609 amino acid residues and 503 amino acid residues in length, respectively, sharing 60% overall sequence identity. These proteins also share

434 40% sequence identity to the distal long tail fiber RBP of *Cronobacter* phage
435 vB_CsaM_GAP161 [YP_006986537.1] and are homologs to the long tail fiber RBP
436 Gp37 of phage T4 [AJC64544.1]. An alignment of these two proteins reveals a high
437 degree of conservation at the N-terminus associated with the proximal tail fiber, as
438 well as at the C-terminus associated with host recognition (**Fig. S4**). More
439 specifically, the C-terminal 63 amino acids present a 95% sequence identity.
440 Similarly, in the phage TG1 and ϕ R1-RT genomes, *ORF159* codes for the short tail
441 fiber (STF) protein Gp12, both of 446 amino acid residues in length and which are
442 almost identical to each other, sharing 95% overall sequence identity (**Fig. S5**). These
443 proteins are homologous to the STF protein Gp12 of phage T4 [NP_049770.1].

444

445 **DNA Packaging.** In phage TG1, *ORF164* and *ORF165/ORF167* genes code for the
446 small (TerS) and large (TerL) DNA packaging subunits respectively of a phage
447 terminase protein complex (or holoterminease) that initiates, drives, and terminates
448 translocation of phage DNA into proheads (48). The homologous genes in phage
449 ϕ R1-RT are represented by *ORF165* (TerS) and *ORF166/ORF168* (TerL). Usually,
450 *terS* and *terL* are arranged side by side but in phage TG1 and ϕ R1-RT two ORFs
451 homologous with *terL* are found. *ORF165* in TG1 and *ORF166* in ϕ R1-RT show
452 sequence similarity to the N-terminus of the phage T4 TerL. Likewise *ORF167* in
453 TG1 and *ORF168* in ϕ R1-RT show sequence similarity to the C-terminus of phage T4
454 TerL. BLASTX analysis (37, 38) reveals the *terL* gene in both phages is interrupted
455 by a transposase (PHA02552). Additionally, during packaging, the DNA ends are also
456 protected against host RecBCD nuclease action by Gp2, the DNA end protector
457 protein (49); identified in phage TG1 and in ϕ R1-RT as the product of *ORF146*.

Homing Endonucleases. Homing endonuclease genes (HEGs) are not genuine phage DNA, but rather belong to intron associated selfish DNA elements (50) and are commonly found interspersed throughout *Myoviridae* genomes (41). Among the HEGs identified in phage TG1, *ORF148* and *ORF232* exhibit similarity to shortened helix-turn-helix (HN-H) endonucleases, and *ORF9*, *ORF43*, and *ORF66* to GIY-YIG group I intron endonucleases. BLASTX analysis (37, 38) reveals Gp47 (recombination-related endonuclease II) is divided by *ORF66* which contains the HEG. Likewise, the gene coding for UvsX is intersected by *ORF43*, which contains the HEG. *ORF232* also divides the *NrdA* gene. In phage ϕ R1-RT five HEGs are also found throughout the genome of which only *ORF148* is homologous to the helix-turn-helix (HN-H) endonuclease that is also located in phage TG1 between Gp4 and Gp53. *ORF20*, *ORF51*, *ORF163*, and *ORF234* exhibit similarity to GIY-YIG group I intron endonucleases, none of which interrupt or intersect other ϕ R1-RT genes.

Lysis. The final stage of the phage lytic cycle involves the degradation of the bacterial cell wall and release of progeny phages induced by the effect a pore producing protein, the holin, and a peptidoglycan degrading enzyme, the endolysin (51). In TG1, *ORF127* and *ORF122* in ϕ R1-RT each encode an obvious endolysin containing a bacteriophage T4-like lysozyme protein domain (pfam00959) and phage-related muramidase (COG3772). Access of the endolysin to the cell wall occurs through the presence of the holin. Holins are small phage encoded proteins characterized by the presence of TMDs, accumulating in the cytoplasmic membrane during infection until suddenly at a specific time, trigger to form lethal lesions resulting in destruction of the cell wall (51, 52). A search for the TG1 and ϕ R1-RT holins revealed the putative product of their respective *ORF253* gene contains a predicted t-holin domain

(pfam11031) with 70% identity to the phage holin of *Enterobacteria* phage CC31 [YP_004010117.1]. The protein sequences are predicted to contain a single TMD spanning aa interval 30-49, as well as a large C-terminal periplasmic domain spanning the aa residue from position 50 to the end terminal amino acid at position 218; a characteristic bitopic topology found in the holin proteins of T4-like phages (53). Moreover, as in phage T4 the putative holin gene is separated from the endolysin gene. An additional search for *Rz/RzI* genes coding for transmembrane spanins involved in the disruption of the outer membrane of the host was also conducted based on gene arrangement and membrane localization signals (54). The search revealed two candidate genes, *ORF225* and *ORF224* in phage TG1 and *ORF227* and *ORF226* in phage ϕ R1-RT, homologous to phage T4 *pseT.3 (Rz)* and *pseT.2 (RzI)*, respectively. As in phage T4, the *Rz/RzI* genes are adjacent to each other, arranged with overlapping stop and start codons, and additionally no part of the *RzI* sequence is embedded within the *Rz* coding region (54). In TG1 and ϕ R1-RT, *Rz* possesses a single amino-terminal TMD, and *RzI* encodes an outer membrane lipoprotein based on the presence of a signal peptidase II (SPII) cleavage site located between amino acid residues 16 and 17 as predicted by Lipop (54, 55). Lastly, the presence of phage T4 homologs to *rI* lysis inhibition regulator membrane protein and *rIII* lysis inhibitor accessory protein in TG1 and ϕ R1-RT suggest the potential for lysis inhibition (LIN) following superinfection (56, 57).

Phylogeny of TG1. It is interesting to note that very similar bacteriophages with an overall DNA sequence identity of 91% were isolated from such different locations and sources, as phages TG1 and ϕ R1-RT were isolated in Canada from pig manure, and in Finland from raw sewage, respectively. Moreover, less than 34% overall DNA

similarity exists with their closest neighbours within the *Myoviridae* (**Table S9**). The relatedness of these two phages was further explored using progressiveMauve (**Fig. 2**) (58, 59); CoreGenes (60, 61) which the Bacterial and Archaeal Virus Subcommittee of the International Committee on Taxonomy of Viruses (ICTV) has extensively used to compare the proteomes of viruses; and by phylogenetic analysis of their whole genome sequences (**Fig. S6**) and their large terminase subunit protein sequences (**Fig. 3**). It is evident from phylogenetic analyses that TG1 and ϕ R1-RT form a distinct taxonomic clade among their closest neighbours. Based on these observations and using a 95% DNA sequence identity as the criterion for demarcation for a species, a new genus named *Tg1virus* with phages TG1 and ϕ R1-RT as member species was proposed to the ICTV (approved in 2016 and pending ratification).

Growth curves. In order to study the efficiency of phage infection at different temperatures bacterial growth after phage infection with ϕ R1-RT was measured. Host bacterial strain was grown at selected temperatures with addition of different phage stock dilutions. Bacterial growth was followed for 3 d at 4°C, 2d at 10°C and 16°C and 1 d at 22°C and 37°C. Lysis of the bacterial cultures was observed at 4°C, 10°C, 16°C and 22°C, whereas at 37°C the bacteria were not significantly affected even with the highest initial phage concentrations (**Fig. 4**, panels A). The onset time of lysis depended on the temperature and initial phage titer. At 4°C the bacterial culture started to lyse after 56-60 h, at 10°C already at 16 h with highest phage titer and at 24-28 h with the lowest phage titer. The corresponding times for 16°C and 22°C were 6 and 12 h. While the lysis at 10°C and 16°C was complete, at 22°C strong regrowth after the initial lysis took place. At 4°C the 3 d incubation time was not long enough to follow the lysis to completion. Under all tested conditions negative (medium only)

controls showed no increase in the absorbance, whereas the positive (bacteria only) controls presented the normal bacterial growth pattern.

Transduction. To study the transducing potential of ϕ R1-RT, transduction of the Km^R and urease-negative phenotype of strain YeO3-*hfq*::Km to the Km^S and urease-positive wild type strain 64741/76 was assayed. Repression of urease activity is one of the phenotypes of the *hfq* mutant (62) and could be used to confirm the transduction of the *hfq*::Km allele. The transduction assays were performed in 10 parallel tubes using a MOI of 0.006. A total of 6.6×10^8 PFU from the transducing lysate resulted in a total of 3 Km^R urease-negative colonies that were confirmed by PCR. From this the calculated transduction frequency in the experiment was 4.5×10^{-7} transductants per PFU.

Identification of the phage receptors – pseudolysogeny. As the LPS and protein profiles of the phage resistant mutants YeO3- ϕ R1-RT-2, YeO3- ϕ R1-RT-7, and YeO3- ϕ R1-RT-9 did not differ from those of the wild type bacteria (data not shown) the genomic DNA of the mutant and the wild type strains were sequenced. The *de novo* assembly results showed that the total scaffold sizes of the assembled genomes of the three mutants were ~165-173 kb larger than that of the wild type parental strain (**Table S10**). This suggested that the mutants carried extra DNA and the size matched very close to the size of phage ϕ R1-RT genome (168,809 bp). This immediately raised the possibility that the phage had lysogenized the host and would reside as a prophage. In all three draft genomes the phage genome sequence was indeed identified and in all it formed the scaffold 4.1 with almost identical sizes (**Table S10**). Significantly, in all three cases the scaffold sequences were 100% identical to phage

558 ϕ R1-RT sequence without any flanking host sequences, suggesting that the phage
559 genome resided in these bacteria as an autonomous replicating unit in a state known
560 as pseudolysogeny. Such state has been described for T4-like phages (63).

561
562 **Identification of the phage receptors – transposon insertion library screening.** As
563 selection of spontaneous phage resistant mutants seemed to favor pseudolysogeny we
564 decided to use a different approach. A *CatMu*-transposon library of strain YeO3-R1
565 (26) was exposed the ϕ R1-RT for 2 hr and the surviving phage-resistant mutants were
566 grown on LA-CIm plates. The recovered colonies were tested to be true ϕ R1-RT
567 resistant mutants. In order to exclude pseudolysogens, the clones were screened with
568 ϕ R1-RT specific PCR, and negative ones were further analysed by *CatMu*-specific
569 arbitrary PCR to identify the CatMu insertion site (26). For four of the candidates the
570 transposon insertion site was identified as gene *Y11_04441* of the *Y. enterocolitica*
571 O:3 strain Y11 genome (NC_017564.1). In strain Y11 genome the gene was
572 annotated to encode for the outer membrane porin OmpC, however, in all other *Y.*
573 *enterocolitica* genomic sequences as OmpF, therefore we opted to use OmpF. To
574 confirm that OmpF is the ϕ R1-RT receptor, one of the mutants YeO3-R1-Cat17 was
575 complemented with the wild type *ompF* gene either *in trans* with plasmid
576 pTM100_OmpF or *in cis* by suicide plasmid pSW25T_OmpF. Both of these
577 approaches resulted in regaining the phage sensitivity thus confirming that OmpF
578 serves as ϕ R1-RT receptor.

579
580 **The LPS inner core heptose region functions as a receptor.** Adsorption
581 experiments were carried out to study the ability of ϕ R1-RT and TG1 to interact with
582 *Y. enterocolitica* O:3 derivatives differing mainly in their LPS composition (**Fig. 5**). A

short 5 min adsorption time was used as it produced highest resolution between the strains. A general observation was that TG1 adsorbed faster than ϕ R1-RT. Both phages showed negligible adsorption to YeO3-c-R1-Cat17, the *ompF* mutant strain and adsorbed well to both *ompF*-complemented strains. Both phages showed reduced but clear adsorption to the pseudolysogen, indicating changes in abundance or exposure of the phage receptor(s). Finally, the adsorption to the inner core mutants decreased with the truncation of the core oligosaccharide suggesting that the inner core heptoses are part of the secondary receptor (**Fig. 5**).

Temperature-dependence of *ompF* expression. We then wondered whether the temperature-dependent sensitivity of *Y. enterocolitica* O:3 could be due to *ompF* regulation. The expression of *ompF* under different growth temperatures was analysed from RNA-sequencing and quantitative proteomics (LC-MS/MS) data. The transcriptomic data showed an inverse correlation between the expression of *ompF* and the temperature of incubation (**Fig. 4**, panels B). Consistently, the quantitative proteomics demonstrated much higher abundance of the OmpF protein in the 22°C sample when compared to the 37°C sample, where the abundance barely exceeded the threshold of identification (**Fig. 4**, panels B).

In vitro expression of the long tail fiber protein Gp37 of phage TG1. In this study, co-expression with the phage encoded chaperones Gp38 (required for oligomerization) and Gp57A, which is also thought to participate in assembly (64, 65) was utilized in an attempt to synthesize the native form of distal long tail fiber protein of phage TG1 as previously described for the production of Gp37 from phage T4 (28). SDS-PAGE demonstrated that an oligomer of approximately 210 kDa was obtained

when Gp37 was co-expressed with Gp38 in a bicistronic plasmid (pCDF-Duet-1 Gp37-Gp38) or when this same plasmid was co-expressed with Gp57A (**Fig. S7**, lanes 3 and 5). Under reduced conditions Gp37 appears as a monomer of approximately 70 kDa in size (**Fig. S7**, lanes 4 and 6). This estimate is close to the predicted molecular mass of the recombinant phage TG1 Gp37 determined via MS at approximately 68.050 kDa. Peptide mass fingerprinting confirmed the identity of the protein (**Fig. S8**). Based on the protein expression results obtained, it appears that in phage TG1 only the Gp38 chaperone is essential and the general chaperone Gp57A is not required for *in-vitro* protein folding of Gp37 as has been reported for phage T4 (28). The formation of higher molecular weight oligomers of phage TG1 Gp37 is consistent with previous reports that describe RBPs of phages present as homotrimers in solution migrating in the SDS-PAGE with a mobility that corresponds to that of oligomeric forms (28, 66–68).

Confirmation of host binding specificity. Host binding specificity was then tested through immunolabeling of bacterial cells with phage TG1 LTF protein Gp37 followed by detection with anti-His₆ antibodies and DyLight 488 conjugated secondary antibodies. Consistent with the temperature dependent infection of phage TG1, the application of the LTF protein Gp37 to *Y. enterocolitica* cells showed decoration of the surface of *Y. enterocolitica* O:3, O:5,27, and O:9 cells when these were grown at 25°C but not at 37°C (**Fig. 6**). Notably, binding was more apparent near the apex of the cells which is also reported to occur in other phages such as λ , T4, T7, KVP40 and ϕ A1122, preferentially infecting cells at the poles (69).

DISCUSSION

Among bacteriophages, the C-terminus of RBPs involved in ligand interactions usually exhibits considerable sequence divergence, thus providing diversity in host specificity. In the case of ϕ R1-RT and TG1, the high sequence identity at the C-terminus of their long tail fiber and short tail fiber proteins may account for the striking similarity in virulence of these two phages for *Y. enterocolitica*. Notably, phage ϕ R1-RT shows virulence to strains of the same serotypes as phage TG1. Based on adsorption experiments, the outer membrane protein OmpF and the inner core heptosyl residues of the LPS serve as phage receptors for phage TG1 and ϕ R1-RT. It is worth noting however, that the *E. coli* strain DH10B/pTM100_OmpF was not sensitive to ϕ R1-RT. We reasoned that this could be due to poor expression of *Y. enterocolitica* OmpF in *E. coli* or more likely that the LPS inner core, known to be used by T4-like phages as the secondary receptor (76, 77) was not compatible. The inner core structures of *E. coli* and *Y. enterocolitica* differ substantially potentially explaining this result.

Multiple lines of evidence suggest OmpF is the primary host range determinant for these two bacteriophages. First, a multiple alignment of OmpF amino acid sequences of *Y. enterocolitica* (from a BLASTP search of sequence databases using the O:3 OmpF sequence as query) suggest the restricted host range of these phages among *Y. enterocolitica* serotypes could be due to OmpF. The alignment provided a distribution of conserved amino acid residues and the presence of regions with high and low homologies, which coincide with eight transmembrane domains and eight “external” loops, respectively of the topology of the OmpF porin from *E. coli* (70, 71). The search and alignment of the sequences (**Fig. S9**) revealed that the OmpF sequences of the ϕ R1-RT sensitive serotypes are 100% identical. The most dramatic differences

658 between the serotypes map to loop 4. In the alignment most close to the O:3 sequence
 659 is the serotype O:7,8,19 OmpF that is 96% identical to O:3 and may still be sensitive
 660 to ϕ R1-RT; in it the loop 4 sequence differs least, while in others differences are
 661 bigger and also accumulate in other loops, mainly in loops 5, 6, and 7 (**Fig. S9**). The
 662 porin loops are plausible binding sites for bacteriophages as demonstrated by the
 663 interaction of *E. coli* OmpF and K20 phages which bind to the L5, L6, and L7
 664 external loops (72–74). Thus it is likely that the loop 4 sequence is targeted by the
 665 ϕ R1-RT or TG1 receptor binding proteins, however, experimental evidence is
 666 necessary to confirm this. Secondly, RNA-sequencing and quantitative proteomics
 667 data, the analysis of growth curves of *Y. enterocolitica* infected with ϕ R1-RT at
 668 various temperatures (4°C to 37°C), as well as phage host range analysis results
 669 conducted at 25°C and 37°C clearly indicate that the failure of ϕ R1-RT and TG1 to
 670 infect *Y. enterocolitica* O:3 at 37°C is due to the strong repression of the *ompF* gene.
 671 The temperature dependent expression of OmpF results also agree with a previous
 672 study, where two-dimensional gel electrophoresis of whole-cell proteins of *Y.*
 673 *enterocolitica* cultured at 25°C and 37°C suggested that OmpF is downregulated
 674 when the bacteria were cultured at 37°C (75). Consistent with this observation, the
 675 application of immunolabelled phage TG1 receptor binding protein Gp37 to *Y.*
 676 *enterocolitica* cells showed decoration of the surface of *Y. enterocolitica* O:3, O:5,27,
 677 and O:9 cells when these were cultured at 25°C but not at 37°C. The decoration of the
 678 cell surface agrees with a high level expression of this major outer membrane protein
 679 class depending on the bacterial species and the environmental conditions, which can
 680 reach about 10^4 – 10^6 copies per cell (72). It is reasonable to suggest then that the
 681 phage TG1 distal long tail fiber protein Gp37 (and by extension, its homolog in ϕ R1-
 682 RT) is specifically involved in binding to OmpF while presumably, the short tail fiber

protein Gp12 binds to the inner core of LPS, as is reported to occur in other T even phages as a secondary receptor (76, 77).

The *in vitro* temperature dependent infection of these two highly related phages, questions their potential use as biocontrol or therapeutic agents as has been suggested for the temperate *Yersinia* phage PY100 (18, 78). On the other hand, it is not known whether the *ompF* gene is expressed *in vivo* justifying further studies towards finding that out. However, due to their marked specificity for the epidemiological relevant *Y. enterocolitica* serotypes O:3, O:5,27, and O:9, these phages may prove useful for diagnostic purposes. In addition, the successful synthesis of the long tail fiber of phage TG1 opens up the possibility of its use as a probe as well as for the production of suitable amounts of protein for X-ray crystallography to elucidate its atomic structure or co-crystallization with its receptor OmpF to shed light on specific host cell receptor-virus interactions.

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708 REFERENCES

709

- 710 1. **Bottone EJ.** 1999. *Yersinia enterocolitica*: overview and epidemiologic
711 correlates. *Microbes Infect* **1**:323–333.
- 712 2. **Fredriksson-Ahomaa M, Stolle A, Korkeala H.** 2006. Molecular
713 epidemiology of *Yersinia enterocolitica* infections. *FEMS Immunol Med*
714 *Microbiol* **47**:315–329.
- 715 3. **Fukushima H, Shimizu S, Inatsu Y.** 2011. *Yersinia enterocolitica* and
716 *Yersinia pseudotuberculosis* Detection in Foods. *J Pathog* **2011**:735308.
- 717 4. **McNally A, Thomson NR, Reuter S, Wren BW.** 2016. “Add, stir and
718 reduce”: *Yersinia* spp. as model bacteria for pathogen evolution. *Nat Rev*
719 *Microbiol* **14**:177–190.
- 720 5. **Verhaegen J, Charlier J, Lemmens P, Delmee M, Van Noyen R, Verbist**
721 **L, Wauters G.** 1998. Surveillance of human *Yersinia enterocolitica*
722 infections in Belgium: 1967-1996. *Clin Infect Dis* **27**:59–64.
- 723 6. **Wren BW.** 2003. The yersiniae—a model genus to study the rapid evolution
724 of bacterial pathogens. *Nat Rev* **1**:55–64.
- 725 7. **Wauters G, Kandolo K, Janssens M.** 1987. Revised biogrouping scheme of
726 *Yersinia enterocolitica*. *Contrib Microbiol Immunol* **9**:14–21.
- 727 8. **Aussel L, Therisod H, Karibian D, Perry MB, Bruneteau M, Caroff M.**
728 2000. Novel variation of lipid A structures in strains of different *Yersinia*
729 species. *FEBS Lett* **465**:87–92.
- 730 9. **Bruneteau M, Minka S.** 2003. Lipopolysaccharides of bacterial pathogens
731 from the genus *Yersinia*: a mini-review. *Biochimie* **85**:145–152.
- 732 10. **ECDC.** 2015. Surveillance of seven priority food- and waterborne diseases
733 in the EU/EEA. Stockholm, Sweden.
- 734 11. **Kiljunen S, Vilen H, Pajunen M, Savilahti H, Skurnik M.** 2005.
735 Nonessential genes of phage phiYe03-12 include genes involved in
736 adaptation to growth on *Yersinia enterocolitica* serotype O:3. *J Bacteriol*
737 **187**:1405–1414.
- 738 12. **Pajunen M, Kiljunen S, Skurnik M.** 2000. Bacteriophage phiYe03-12,
739 specific for *Yersinia enterocolitica* serotype O:3, is related to coliphages T3
740 and T7. *J Bacteriol* **182**:5114–5120.
- 741 13. **Pajunen MI, Kiljunen SJ, Söderholm ME, Skurnik M, Soderholm ME,**
742 **Skurnik M.** 2001. Complete genomic sequence of the lytic bacteriophage

- 743 phiYe03-12 of *Yersinia enterocolitica* serotype O:3. J Bacteriol **183**:1928–
744 1937.
- 745 14. **Leon-Velarde CG, Kropinski AM, Chen S, Abbasifar A, Griffiths MW,**
746 **Odumeru JA.** 2014. Complete genome sequence of bacteriophage
747 vB_YenP_AP5 which infects *Yersinia enterocolitica* of serotype O:3. Virol J
748 **11**:188.
- 749 15. **Hertwig S, Klein I, Schmidt V, Beck S, Hammerl JA, Appel B.** 2003.
750 Sequence analysis of the genome of the temperate *Yersinia enterocolitica*
751 phage PY54. J Mol Biol **331**:605–622.
- 752 16. **Kiljunen S, Hakala K, Pinta E, Huttunen S, Pluta P, Gador A, Lonnberg**
753 **H, Skurnik M.** 2005. Yersiniophage phiR1-37 is a tailed bacteriophage
754 having a 270 kb DNA genome with thymidine replaced by deoxyuridine.
755 Microbiology **151**:4093–4102.
- 756 17. **Skurnik M, Hyytiäinen HJ, Happonen LJ, Kiljunen S, Datta N, Mattinen**
757 **L, Williamson K, Kristo P, Szeliga M, Kalin-Mänttari L, Ahola-Iivarinen**
758 **E, Kalkkinen N, Butcher SJ.** 2012. Characterization of the genome,
759 proteome, and structure of yersiniophage ϕ R1-37. J Virol **86**:12625–42.
- 760 18. **Schwudke D, Ergin A, Michael K, Volkmar S, Appel B, Knabner D,**
761 **Konietzny A, Strauch E.** 2008. Broad-host-range *Yersinia* phage PY100:
762 genome sequence, proteome analysis of virions, and DNA packaging
763 strategy. J Bacteriol **190**:332–342.
- 764 19. **Skurnik M.** 1999. Genetics of Bacterial Polysaccharides, p. 23–51.. *In*
765 Goldberg, J (ed.), . CRC Press, Boca Raton, FL.
- 766 20. **Al-Hendy A, Toivanen P, Skurnik M.** 1991. Expression cloning of *Yersinia*
767 *enterocolitica* O:3 rfb gene cluster in *Escherichia coli* K12. Microb Pathog
768 **10**:47–59.
- 769 21. **Baker PM, Farmer JJ.** 1982. New bacteriophage typing system for *Yersinia*
770 *enterocolitica*, *Yersinia kristensenii*, *Yersinia frederiksenii*, and *Yersinia*
771 *intermedia*: correlation with serotyping, biotyping, and antibiotic
772 susceptibility. J Clin Microbiol **15**:491–502.
- 773 22. **Al-Hendy A, Toivanen P, Skurnik M.** 1992. Lipopolysaccharide O side
774 chain of *Yersinia enterocolitica* O:3 is an essential virulence factor in an
775 orally infected murine model. Infect Immun **60**:870–875.
- 776 23. **Skurnik M.** 1984. Lack of correlation between the presence of plasmids
777 and fimbriae in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. J
778 Appl Bacteriol **56**:355–363.
- 779 24. **Kutter E.** 2009. Phage Host Range and Efficiency of Plating, p. 141. *In*
780 Clokie, M, Kropinski, AM (eds.). Humana Press.
- 781 25. **Biedzka-Sarek M, Venho R, Skurnik M.** 2005. Role of YadA, Ail, and

782 Lipopolysaccharide in Serum Resistance of *Yersinia enterocolitica* Serotype
783 O:3. Infect Immun **73**:2232–2244.

784 26. **Pinta E, Li Z, Batzilla J, Pajunen M, Kasanen T, Rabsztyk K, Rakin A,**
785 **Skurnik M.** 2012. Identification of three oligo-polysaccharide-specific
786 ligases in *Yersinia enterocolitica*. Mol Microbiol **83**:125–36.

787 27. **Pajunen M, Kiljunen S, Skurnik M.** 2012. Construction and Screening of a
788 Transposon Insertion Library of *Yersinia enterocolitica* (YeO3-R1). Bio-
789 protocol **2**.

790 28. **Bartual SG, Garcia-Doval C, Alonso J, Schoehn G, van Raaij MJ.** 2010.
791 Two-chaperone assisted soluble expression and purification of the
792 bacteriophage T4 long tail fibre protein Gp37. Protein Expr Purif **70**:116–
793 121.

794 29. **Laemmli UK.** 1970. Cleavage of structural proteins during the assembly of
795 the head of bacteriophage T4. Nature **227**:680–5.

796 30. **Javed MA, Poshtiban S, Arutyunov D, Evoy S, Szymanski CM.** 2013.
797 Bacteriophage receptor binding protein based assays for the simultaneous
798 detection of *Campylobacter jejuni* and *Campylobacter coli*. PLoS One
799 **8**:e69770.

800 31. **Schneider CA, Rasband WS, Eliceiri KW.** 2012. NIH Image to ImageJ: 25
801 years of image analysis. Nat Methods **9**:671–5.

802 32. **Daligault HE, Davenport KW, Minogue TD, Bishop-Lilly KA, Broomall**
803 **SM, Bruce DC, Chain PS, Coyne SR, Frey KG, Gibbons HS, Jaissle J,**
804 **Koroleva GI, Ladner JT, Lo C-C, Munk C, Palacios GF, Redden CL,**
805 **Rosenzweig CN, Scholz MB, Johnson SL.** 2014. Whole-Genome *Yersinia*
806 sp. Assemblies from 10 Diverse Strains. Genome Announc **2**.

807 33. **Brenner DJ, Ursing J, Bercovier H, Steigerwalt AG, Fanning GR, Alonso**
808 **JM, Mollaret HH.** 1980. Deoxyribonucleic acid relatedness in *Yersinia*
809 *enterocolitica* and *Yersinia enterocolitica*-like organisms. Curr Microbiol **4**.

810 34. **Schattner P, Brooks AN, Lowe TM.** 2005. The tRNAscan-SE, snoscan and
811 snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids
812 Res **33**:W686–9.

813 35. **Laslett D, Canback B.** 2004. ARAGORN, a program to detect tRNA genes
814 and tmRNA genes in nucleotide sequences. Nucleic Acids Res **32**.

815 36. **Miller ES, Kutter E, Mosig G, Arisaka F, Kunisawa T, Rüger W.** 2003.
816 Bacteriophage T4 genome. Microbiol Mol Biol Rev **67**:86–156.

817 37. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local
818 alignment search tool. J Mol Biol **215**:403–10.

819 38. **Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W,**

820 **Lipman DJ**. 1997. Gapped BLAST and PSI-BLAST: a new generation of
821 protein database search programs. *Nucleic Acids Res* **25**:3389–402.

822 39. **Manosas M, Perumal SK, Croquette V, Benkovic SJ**. 2012. Direct
823 observation of stalled fork restart via fork regression in the T4 replication
824 system. *Science* **338**:1217–20.

825 40. **Liu J, Morrical SW**. 2010. Assembly and dynamics of the bacteriophage T4
826 homologous recombination machinery. *Virol J* **7**:357.

827 41. **Petrov VM, Ratnayaka S, Nolan JM, Miller ES, Karam JD**. 2010. Genomes
828 of the T4-related bacteriophages as windows on microbial genome
829 evolution. *Virol J* **7**:292.

830 42. **Lundin D, Torrents E, Poole AM, Sjöberg B-M**. 2009. RNRdb, a curated
831 database of the universal enzyme family ribonucleotide reductase, reveals
832 a high level of misannotation in sequences deposited to Genbank. *BMC*
833 *Genomics* **10**:589.

834 43. **Lavigne R, Sun WD, Volckaert G**. 2004. PHIRE, a deterministic approach
835 to reveal regulatory elements in bacteriophage genomes. *Bioinformatics*
836 **20**.

837 44. **Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li**
838 **WW, Noble WS**. 2009. MEME SUITE: tools for motif discovery and
839 searching. *Nucleic Acids Res* **37**:W202–8.

840 45. **Gautheret D, Lambert A**. 2001. Direct RNA motif definition and
841 identification from multiple sequence alignments using secondary
842 structure profiles. *J Mol Biol* **313**:1003–11.

843 46. **Macke TJ, Ecker DJ, Gutell RR, Gautheret D, Case DA, Sampath R**. 2001.
844 RNAMotif, an RNA secondary structure definition and search algorithm.
845 *Nucleic Acids Res* **29**:4724–35.

846 47. **Leiman PG, Arisaka F, van Raaij MJ, Kostyuchenko VA, Aksyuk AA,**
847 **Kanamaru S, Rossmann MG**. 2010. Morphogenesis of the T4 tail and tail
848 fibers. *Virol J* **7**:355.

849 48. **Mitchell MS, Rao VB**. 2006. Functional analysis of the bacteriophage T4
850 DNA-packaging ATPase motor. *J Biol Chem* **281**:518–27.

851 49. **Lipinska B, Rao AS, Bolten BM, Balakrishnan R, Goldberg EB**. 1989.
852 Cloning and identification of bacteriophage T4 gene 2 product Gp2 and
853 action of Gp2 on infecting DNA in vivo. *J Bacteriol* **171**:488–97.

854 50. **Bell-Pedersen D, Quirk S, Clyman J, Belfort M**. 1990. Intron mobility in
855 phage T4 is dependent upon a distinctive class of endonucleases and
856 independent of DNA sequences encoding the intron core: mechanistic and
857 evolutionary implications. *Nucleic Acids Res* **18**:3763–70.

- 858 51. **Wang IN, Smith DL, Young R.** 2000. Holins: the protein clocks of
859 bacteriophage infections. *Annu Rev Microbiol* **54**:799–825.
- 860 52. **Dewey JS, Savva CG, White RL, Vitha S, Holzenburg A, Young R.** 2010.
861 Micron-scale holes terminate the phage infection cycle. *Proc Natl Acad Sci*
862 *U S A* **107**:2219–23.
- 863 53. **Tran TAT, Struck DK, Young R.** 2005. Periplasmic domains define holin-
864 antiholin interactions in T4 lysis inhibition. *J Bacteriol* **187**:6631–40.
- 865 54. **Summer EJ, Berry J, Tran TA, Niu L, Struck DK, Young R.** 2007. Rz/Rz1
866 Lysis Gene Equivalents in Phages of Gram Negative Hosts. *J Mol Biol* **373**.
- 867 55. **Juncker AS, Willenbrock H, Von Heijne G, Brunak S, Nielsen H, Krogh**
868 **A.** 2003. Prediction of lipoprotein signal peptides in Gram-negative
869 bacteria. *Protein Sci* **12**:1652–62.
- 870 56. **Doermann AH.** 1948. Lysis and Lysis Inhibition with *Escherichia coli*
871 Bacteriophage. *J Bacteriol* **55**:257–76.
- 872 57. **Burch LH, Zhang L, Chao FG, Xu H, Drake JW.** 2011. The bacteriophage
873 T4 rapid-lysis genes and their mutational proclivities. *J Bacteriol*
874 **193**:3537–45.
- 875 58. **Darling AC, Mau B, Blattner FR, Perna NT.** 2004. Mauve: multiple
876 alignment of conserved genomic sequence with rearrangements. *Genome*
877 **14**.
- 878 59. **Darling AE, Mau B, Perna NT.** 2010. progressiveMauve: multiple genome
879 alignment with gene gain, loss and rearrangement. *PLoS One* **5**:e11147.
- 880 60. **Zafar N, Mazumder R, Seto D.** 2002. CoreGenes: A computational tool for
881 identifying and cataloging “core” genes in a set of small genomes. *BMC*
882 *Bioinformatics* **3**.
- 883 61. **Turner D, Reynolds D, Seto D, Mahadevan P.** 2013. CoreGenes3.5: a
884 webserver for the determination of core genes from sets of viral and small
885 bacterial genomes. *BMC Res Notes* **6**:140.
- 886 62. **Kakoschke T, Kakoschke S, Magistro G, Schubert S, Borath M,**
887 **Heesemann J, Rossier O.** 2014. The RNA chaperone Hfq impacts growth,
888 metabolism and production of virulence factors in *Yersinia enterocolitica*.
889 *PLoS One* **9**:e86113.
- 890 63. **Łoś M, Węgrzyn G.** 2012. Pseudolysogeny. *Adv Virus Res* **82**:339–49.
- 891 64. **Marusich EI, Kurochkina LP, Mesyanzhinov V V.** 1998. Chaperones in
892 bacteriophage T4 assembly. *Biochemistry Biokhimia* **63**:399–406.
- 893 65. **Matsui T, Griniuvienė B, Goldberg E, Tsugita A, Tanaka N, Arisaka F.**
894 1997. Isolation and characterization of a molecular chaperone, Gp57A, of

- 895 bacteriophage T4. J Bacteriol **179**:1846–1851.
- 896 66. **Garcia-Doval C, van Raaij MJ.** 2012. Structure of the receptor-binding
897 carboxy-terminal domain of bacteriophage T7 tail fibers. Proc Natl Acad
898 Sci USA **109**:9390–9395.
- 899 67. **Cerritelli ME, Wall JS, Simon MN, Conway JF, Steven AC.** 1996.
900 Stoichiometry and domain organization of the long tail-fiber of
901 bacteriophage T4: a hinged viral adhesin. J Mol Biol **260**:767–780.
- 902 68. **Hashemolhosseini S, Stierhof YD, Hindennach I, Henning U.** 1996.
903 Characterization of the helper proteins for the assembly of tail fibers of
904 coliphages T4 and lambda. J Bacteriol **178**:6258–6265.
- 905 69. **Edgar R, Rokney A, Feeney M, Semsey S, Kessel M, Goldberg MB, Adhya
906 S, Oppenheim AB.** 2008. Bacteriophage infection is targeted to cellular
907 poles. Mol Microbiol **68**:1107–16.
- 908 70. **Guzev K V, Isaeva MP, Novikova OD, Solov'eva TF, Rasskazov VA.** 2005.
909 Molecular characteristics of OmpF-like porins from pathogenic *Yersinia*.
910 Biochemistry Biokhimiia **70**:1104–1110.
- 911 71. **Stenkova AM, Isaeva MP, Shubin FN, Rasskazov VA, Rakin A V.** 2011.
912 Trends of the major porin gene (*ompF*) evolution: insight from the genus
913 *Yersinia*. PLoS One **6**:e20546.
- 914 72. **Achouak W, Heulin T, PagÃ's J-M.** 2001. Multiple facets of bacterial
915 porins. FEMS Microbiol Lett **199**:1–7.
- 916 73. **Silverman JA, Benson SA.** 1987. Bacteriophage K20 requires both the
917 OmpF porin and lipopolysaccharide for receptor function. J Bacteriol
918 **169**:4830–3.
- 919 74. **Traurig M, Misra R.** 1999. Identification of bacteriophage K20 binding
920 regions of OmpF and lipopolysaccharide in *Escherichia coli* K-12. FEMS
921 Microbiol Lett **181**:101–8.
- 922 75. **Gu W, Wang X, Qiu H, Luo X, Xiao D, Xiao Y, Tang L, Kan B, Jing H.** 2012.
923 Comparative antigenic proteins and proteomics of pathogenic *Yersinia*
924 *enterocolitica* bio-serotypes 1B/O: 8 and 2/O: 9 cultured at 25°C and 37°C.
925 Microbiol Immunol **56**:583–94.
- 926 76. **Riede I.** 1987. Receptor specificity of the short tail fibres (Gp12) of T-even
927 type *Escherichia coli* phages. Mol Gen Genet **206**:110–5.
- 928 77. **Thomassen E, Gielen G, Schütz M, Schoehn G, Abrahams JP, Miller S,
929 van Raaij MJ.** 2003. The structure of the receptor-binding domain of the
930 bacteriophage T4 short tail fibre reveals a knitted trimeric metal-binding
931 fold. J Mol Biol **331**:361–73.
- 932 78. **Orquera S, Götz G, Hertwig S, Hammerl J, Sparborth D, Joldic A, Alter T.**

- 933 2012. Control of *Campylobacter* spp. and *Yersinia enterocolitica* by virulent
934 bacteriophages. J Mol Genet Med **6**:273–8.
- 935 79. **Noszczyńska M, Kasperkiewicz K, Duda KA, Podhorodecka J, Rabsztyń**
936 **K, Gwizdała K, Świerżko AS, Radziejewska-Lebrecht J, Holst O,**
937 **Skurnik M.** 2015. Serological characterization of the enterobacterial
938 common antigen substitution of the lipopolysaccharide of *Yersinia*
939 *enterocolitica* O:3. Microbiology **161**:219–27.
- 940 80. **Kay BA, Wachsmuth K, Gemski P, Feeley JC, Quan TJ, Brenner DJ.** 1983.
941 Virulence and phenotypic characterization of *Yersinia enterocolitica*
942 isolated from humans in the United States. J Clin Microbiol **17**:128–138.
- 943 81. **Portnoy DA, Falkow S.** 1981. Virulence-associated plasmids from *Yersinia*
944 *enterocolitica* and *Yersinia pestis*. J Bacteriol **148**:877–883.
- 945 82. **Michiels T, Cornelis GR.** 1991. Secretion of hybrid proteins by the
946 *Yersinia* Yop export system. J Bacteriol **173**:1677–1685.
- 947 83. **Demarre G, Guérout A-M, Matsumoto-Mashimo C, Rowe-Magnus DA,**
948 **Marlière P, Mazel D.** 2005. A new family of mobilizable suicide plasmids
949 based on broad host range R388 plasmid (IncW) and RP4 plasmid
950 (IncPalpha) conjugative machineries and their cognate *Escherichia coli*
951 host strains. Res Microbiol **156**:245–55.
- 952 84. **Hu B, Margolin W, Molineux IJ, Liu J.** 2015. Structural remodeling of
953 bacteriophage T4 and host membranes during infection initiation. Proc
954 Natl Acad Sci USA **112**:E4919–28.
- 955 85. **Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F,**
956 **Dufayard J-F, Guindon S, Lefort V, Lescot M, Claverie J-M, Gascuel O.**
957 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist.
958 Nucleic Acids Res **36**:W465–9.
- 959 86. **Kasperkiewicz K, Swierżko AS, Bartłomiejczyk MA, Cedzynski M,**
960 **Noszczyńska M, Duda KA, Michalski M, Skurnik M.** 2015. Interaction of
961 human mannose-binding lectin (MBL) with *Yersinia enterocolitica*
962 lipopolysaccharide. Int J Med Microbiol **305**:544–52.

963

Table 1. Bacterial strains, plasmids and bacteriophages

Strain	Comments	Reference
<i>Y. enterocolitica</i>		
6471/76 (YeO3)	Serotype O:3, wild type. Human stool isolate	(23)
6471/76-c (YeO3-c)	Virulence-plasmid cured derivative of YeO3	(23)
YeO3-φR1-RT-R2	φR1-RT resistant spontaneous derivative of YeO3	This work
YeO3-φR1-RT-R7	φR1-RT resistant spontaneous derivative of YeO3	This work
YeO3-φR1-RT-R9	φR1-RT resistant spontaneous derivative of YeO3	This work
YeO3-R1	(=YeO3-c-R1) Spontaneous rough strain	(22)
YeO3- <i>hfq</i> ::Km	<i>Hfq</i> ::Km-GenBlock, Km ^R . Urease-negative	(Leskinen et al., submitted for publication)
YeO3-R1-Cat17	<i>ompF</i> :: <i>Cat-Mu</i> derivative of YeO3-R1	This work
YeO3-R1-Cat17::pSW25T_OmpF	<i>Cis</i> complemented <i>ompF</i> :: <i>Cat-Mu</i> strain	This work
YeO3-R1-M164	<i>waaF</i> :: <i>Cat-Mu</i> . derivative of YeO3-R1. Clm ^R	(79)
YeO3-R1-M196	<i>galU</i> :: <i>Cat-Mu</i> derivative of YeO3-R1. Clm ^R	(79)
YeO3-R1-M205	<i>hldE</i> :: <i>Cat-Mu</i> derivative of YeO3-R1. Clm ^R	(79)
YeO3-c-OC		(25)
YeO3-c-OCR		(25)
K14	Serotype O:9	
gc815-73	Serotype O:5,27	(80)
8081	Serotype O:8	(81)
<i>Escherichia coli</i>		
BL21 Star TM (DE3) PLysS		Invitrogen
DH10B		
Plasmids		
pTM100		(82)
pTM100_OmpF	Complementation plasmid with wild type <i>ompF</i> gene	This work

	cloned into pTM100	
pSW25T	Suicide vector	(83)
pSW25T_OmpF	Complementation suicide plasmid with wild type <i>ompF</i> gene cloned into pSW25T	This work
pCDF Duet-1	pCloDF13 replicon, T7lac promoter and 2 MCS sites each with an optional N-terminal His ₆ tag sequence. Streptomycin resistance marker.	Novagen
pET21a(+)	ColE1 (pBR322) replicon, T7lac promoter, N-terminal T7 tag sequence and optional C-terminal His ₆ Tag sequence. Ampicillin resistance marker.	Novagen
pCDF Duet-1 Gp37	Phage TG1 <i>ORF250</i> (4-1,830 bp) cloned in frame into MCS1 of pCDF Duet-1 for expression of N-terminal His ₆ tagged protein Gp37.	This study
pCDF Duet-1 Gp37-Gp38	Phage TG1 <i>ORF251</i> cloned into MCS2 of pCDF Duet-1 Gp37 for co-expression of N-terminal His ₆ tagged Gp37 and tail fiber assembly chaperone Gp38.	This study
pET21a(+) Gp57A	Phage TG1 <i>ORF143</i> cloned into MCS of pET21a(+) for expression of general trimerization chaperone Gp57A.	This study
<hr/> Bacteriophages		
TG1		This study
φR1-RT		This study

Table 2. Lytic activities of phages TG1 and ϕ R1-RT. The sensitivity was tested on 160 *Yersinia* species strains (Table S1) at 25°C.

<i>Yersinia</i> species	Phage sensitive serotypes ^a	Serotypes with phage sensitive (S) and resistant (R) strains	Phage resistant serotypes ^b
<i>Y. enterocolitica</i>	O:1[2], O:2 [2], O:3 [16], O:5 [9], O5,27 [10], O:6 [2], O:7,8 [2], O:9 [13],	O:6,30 [1S/2R], O:6,31 [1S/1R]	O:1,2,3 [1], O:4 [1], O:4,32 [1], O:8 [14], O:10 [4], O:13 [1], O:13a,13b [1], O:13,7 [2], O13,18 [1], O:14 [1], O:20 [2], O:21 [3], O:25 [1], O:25,26,44 [1], O:26,44 [1], O28,50 [1], O:34 [1], O:35,36 [1], O35,52 [1], O:41(27),K1 [1], O41(27),42 [1], O:41(27),42,K1 [1], O:41,43 [1], O:41(27),43 [2], O:50 [1], K1 NT[2], NT[3]
<i>Y. aleksiciae</i>			O:16 [2]
<i>Y. aldovae</i>			UT [2]
<i>Y. bercovieri</i>			O:58,16 [2], NT [1], UT[2]
<i>Y. frederiksenii</i>			O:3 [1], O:16 [1], O:35 [1], O:48 [1], K1 NT[1], NT[1], UT[2]
<i>Y. intermedia</i>			O16,21 [1], O:52,54 [1], UT[2]
<i>Y. kristensenii</i>			O:3 [1], O:12,25 [1], NT[2], UT[3]
<i>Y. mollareti</i>			O:3 [1], O:59(20,36,7) [1], UT[2]
<i>Y. nurmii</i>			UT[1]
<i>Y. pekkanenii</i>			UT[1]
<i>Y. pseudotuberculosis</i>			I [2], O:1b [2], O:3 [2]
<i>Y. rohdei</i>			UT[2]
<i>Y. ruckeri</i>			NT[1], UT[5]

^aThe number of strains studied is given in brackets. Phage ϕ R1-RT sensitivity was tested only with the 109 UH-source strains (Table S1).

^bNT, non-typeable and either cross-reacting or not agglutinating with *Y. enterocolitica* O:3, O:5, O:8 or O:9 antisera. UT, untyped.

FIGURE LEGENDS

Figure 1. Bacteriophage ϕ R1-RT and TG1 morphology by electron microscopy. **Panel A.** ϕ R1-RT virions at 39,440x magnification. The virion head and tail are indicated, as well as long tail fibers (LTF) and a baseplate with protruding tail pins (B). Scale bar, 100 nm. **Panel B.** A ϕ R1-RT virion at 84,320x magnification. A baseplate with protruding tail pins (B), and a neck and collar with neck fibers (A) can be observed. Scale bar, 50 nm. **Panel C.** A ϕ R1-RT virion at 108,800x magnification. Suggested long tail fibers (LTF) can be seen bent up towards the head along the tail sheath, as described for bacteriophage T4 (84). Scale bar, 50 nm. **Panel D.** Bacteriophage TG1 virion shown at 150,000x magnification. A neck and collar with neck fibers (A), a baseplate with protruding tail pins (B), and an extended long tail fiber (LTF) can be observed. Scale bar indicates size in nm.

Figure 2. ProgressiveMauve alignment of phage TG1 and ϕ R1-RT. The genome of ϕ R1-RT [HE956709] is indicated on the top and that of TG1 [KP202158] is shown in the bottom of the figure. The degree of sequence similarity between regions is given by a similarity plot within the coloured blocks with the height of the plot proportional to the average nucleotide identity. Below these are illustrated the phage genes as outlined boxes on the plus (above horizontal) and minus (below horizontal) strands.

Figure 3. Phylogenetic analysis of the large terminase subunit protein sequences of phages TG1, ϕ R1-RT and related bacteriophages. The phylogenetic analysis was constructed using “one click” at phylogeny.fr using MUSCLE for multiple alignment and PhyML for phylogeny (85).

Figure 4. Phage ϕ R1-RT does not propagate at 37°C. **Panel A.** The growth curves of *Y. enterocolitica* infected with ϕ R1-RT. Bacteria were cultured with different concentrations of phage particles in LB at 4°C, 10°C, 16°C, 22°C, and 37°C. Each graph represents the average of five replicates. Note the different scales used for the X-axis at different temperatures. **Panel B.** Analysis of the *ompF* gene expression (left) and protein abundance (right) at different temperatures. The mean expression levels of the *ompF* gene were obtained from RNA-sequencing analysis. The production levels of the OmpF protein was obtained from normalized mean spectral values for the proteins detected by the LC-MS/MS analysis. Error bars represent the calculated standard deviation.

Figure 5. Phages ϕ R1-RT and TG1 use OmpF and LPS inner core heptose region of *Y. enterocolitica* O:3 as receptors. **Panel A.** Adsorption experiments were performed with different LPS and *ompF* mutants, with the complemented strains, and with the pseudolysogen. All strains are OmpF positive with the exception of YeO3-c-R1-Cat17. The TG1 and ϕ R1-RT adsorptions to the bacteria at 5 min are shown as residual PFU percentages. Error bars indicate standard deviations. The no bacteria control (LB) and strains used for adsorptions are indicated below the columns. The LPS chemotypes of the strains are indicated on top of the columns. **Panel B.** The schematic structures of the *Y. enterocolitica* O:3 LPS molecules of different chemotypes (86). Please note that *Y. enterocolitica* O:3 carries simultaneously the S and Ra type LPS molecules. This is indicated in panel A by a plus sign. O-ag, O-antigen or O polysaccharide; OC, outer core hexasaccharide; IC, inner core; LA, lipid A.

Figure 6. Confocal immunofluorescence microscopy images of *Y. enterocolitica* cells after incubation with LTF protein Gp37 derived from phage TG1. Gp37 decorates the cell surface of *Y. enterocolitica* strain K14 of serotype O:9 (a), *Y. enterocolitica* strain gc815-73 of serotype O:5,27 (c), and *Y. enterocolitica* strain 6471/76 of serotype O:3 (e) grown at 25°C, whereas *Y. enterocolitica* strain 8081 of serotype O:8 (g) does not show cell decoration with Gp37. Similar

images to that presented in g were observed when the same strains were grown at 37°C. Differential interference contrast microscopy images of a, c, e and g, are shown in b, d, f and h, respectively. Scale bar represent size in μm .

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Plasmid constructs for chaperone-assisted expression of the distal long tail fiber protein Gp37 of phage TG1. The location of the origins of replication, antibiotic resistance genes (Sm R, streptomycin resistance; Amp R, ampicillin resistance), relevant promoters (T7 lac promoter), Lac I repressor, Multiple cloning sites (yellow) and sequences coding phage Gp37 (red), and chaperones Gp38 and Gp57A (green) are presented.

Figure S2. Map of the phage $\phi\text{R1-RT}$ genome [HE956709]. The genes are shown by different-colored arrows. The arrow direction indicates the coding direction of the genes. The genes encoding putative proteins with an assigned function are shown in black (see also Table S3). The locations of tRNA encoding genes are shown in blue. Hypothetical proteins with an unknown function are depicted in yellow. Homing endonuclease genes are shown in orange. Putative host promoters are shown as pink triangles above the sequence, and putative rho independent terminators are shown as green triangles below the sequence.

Figure S3. Map of the phage TG1 genome [KP202158]. The genes are shown by different-colored arrows. The arrow direction indicates the coding direction of the genes. The genes encoding putative proteins with an assigned function are shown in black (see also Table S2). The locations of tRNA encoding genes are shown in blue. Hypothetical proteins with an unknown function are depicted in yellow. Homing endonuclease genes are shown in orange. Putative host promoters are shown as pink triangles above the sequence, and putative rho independent terminators are shown as green triangles below the sequence.

Figure S4. Multiple sequence alignment of the long tail fiber (Gp37) sequences of phage TG1 and $\phi\text{R1-RT}$. Multiple sequence alignment was performed using Clustal W via Geneious R9 software version 9.0.2. (Biomatters Ltd). Positions which have a single, fully conserved aa residue (100% similarity) are highlighted in black; aa present in 2 of the sequences are highlighted in grey. The homologous phage T4 Gp37 sequence was included in the alignment or comparison.

Figure S5. Multiple sequence alignment of the short tail fiber (Gp12) protein sequences of phage TG1 and $\phi\text{R1-RT}$. Multiple sequence alignment was performed using Clustal W via Geneious R9 software version 9.0.2. (Biomatters Ltd). Positions which have a single, fully conserved aa residue (100% similarity) are highlighted in black; aa present in 2 of the sequences are highlighted in grey. The homologous phage T4 Gp12 sequence was included in the sequence alignment for comparison.

Figure S6. Protein mass fingerprinting of phage TG1 Gp37. The amino acid sequence of phage TG1 Gp37 is shown. Peptide fragments from the analysis of gel slices corresponding to the reduced form of the protein and identified via protein mass fingerprinting are underlined and shown in bold.

Figure S7. Whole genome phylogeny of phages TG1, $\phi\text{R1-RT}$ and related bacteriophages. The phylogenetic tree was generated using using “one click” at phylogeny.fr using MUSCLE for multiple alignment and PhyML for phylogeny (85).

Figure S8. Expression of N-terminal His₆ tagged phage TG1 Gp37. 4-15% Tris-HCl SDS-PAGE run at 4°C, 100V. Lane 1, pCDF-Duet-1 Gp37 (unheated sample); Lane 2, pCDF-Duet-1 Gp37 under reduced conditions (sample heated at 100°C for 10 min in the presence of SDS and β-mercaptoethanol); Lane 3, pCDF-Duet-1 Gp37-Gp38 (unheated sample); Lane 4, pCDF-Duet-1 Gp37-Gp38 reduced conditions; Lane 5, pCDF-Duet-1 Gp37-Gp38 co-expressed with pET21a(+) Gp57A (unheated sample); Lane 6 pCDF-Duet-1 Gp37-Gp38 co-expressed with pET21a(+) Gp57A reduced conditions; Lane 7, pCDF-Duet-1 Gp37 co-expressed with pET21a(+) Gp57A (unheated sample); Lane 8, pCDF-Duet-1 Gp37 co-expressed with pET21a(+) Gp57A reduced conditions; Lane M, molecular weight markers.

Figure S9. CLUSTAL W multiple sequence alignment of the OmpF proteins of *Y. enterocolitica* and related species. The external loops, indicated by brown highlighting and box, were identified based on OmpF alignment of YeO3 OmpF with that of *E. coli*, shown at the bottom. The N-terminal signal-peptide is indicated by blue highlighting and box. Use the zoom-in option to see details of the alignment.

SUPPLEMENTARY TABLES

Table S1. List of *Yersinia* strains used in phage host range experiments

Table S2. Primers used in this work

Table S3. Bacterial strains used to examine the cross infectivity of phage TG1

Table S4. Annotations of bacteriophage TG1 and φR1-RT genes

Table S5. Putative Phage TG1 promoters

Table S6. Putative Phage φR1-RT promoters

Table S7. Predicted terminator sequences of phage TG1

Table S8. Predicted terminator sequences of phage φR1-RT

Table S9. TG1 genome BLASTN analysis

Table S10. Whole genome sequencing statistics after de novo assembly of φR1-RT resistant mutants.

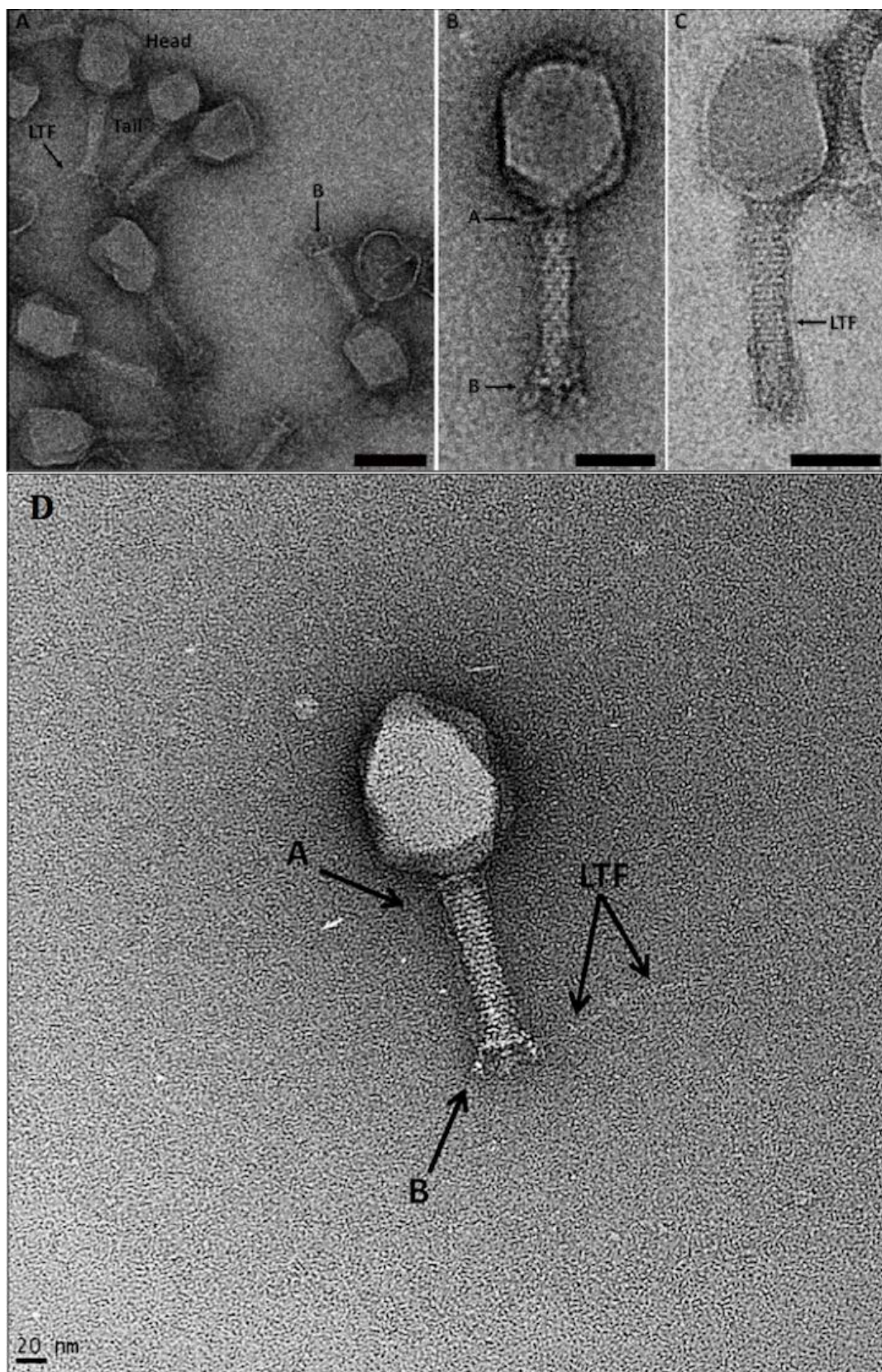


Figure 1.

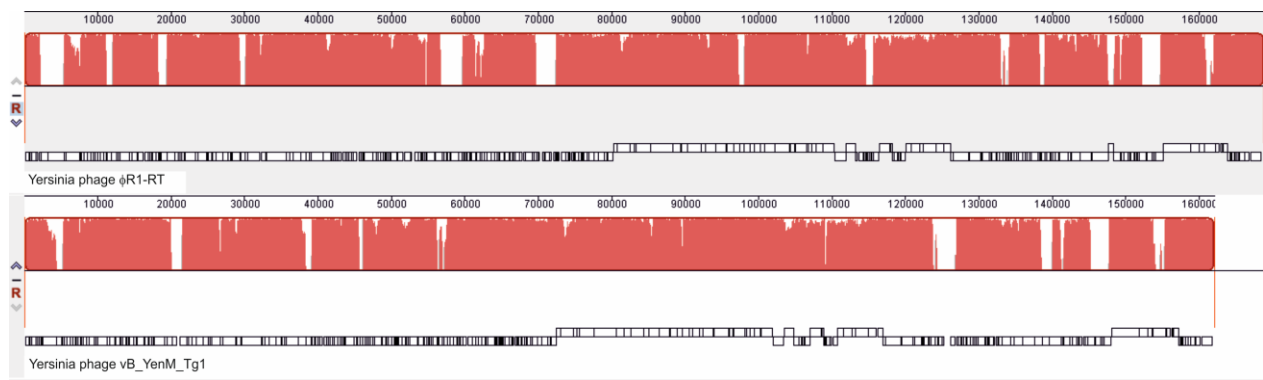


Figure 2.

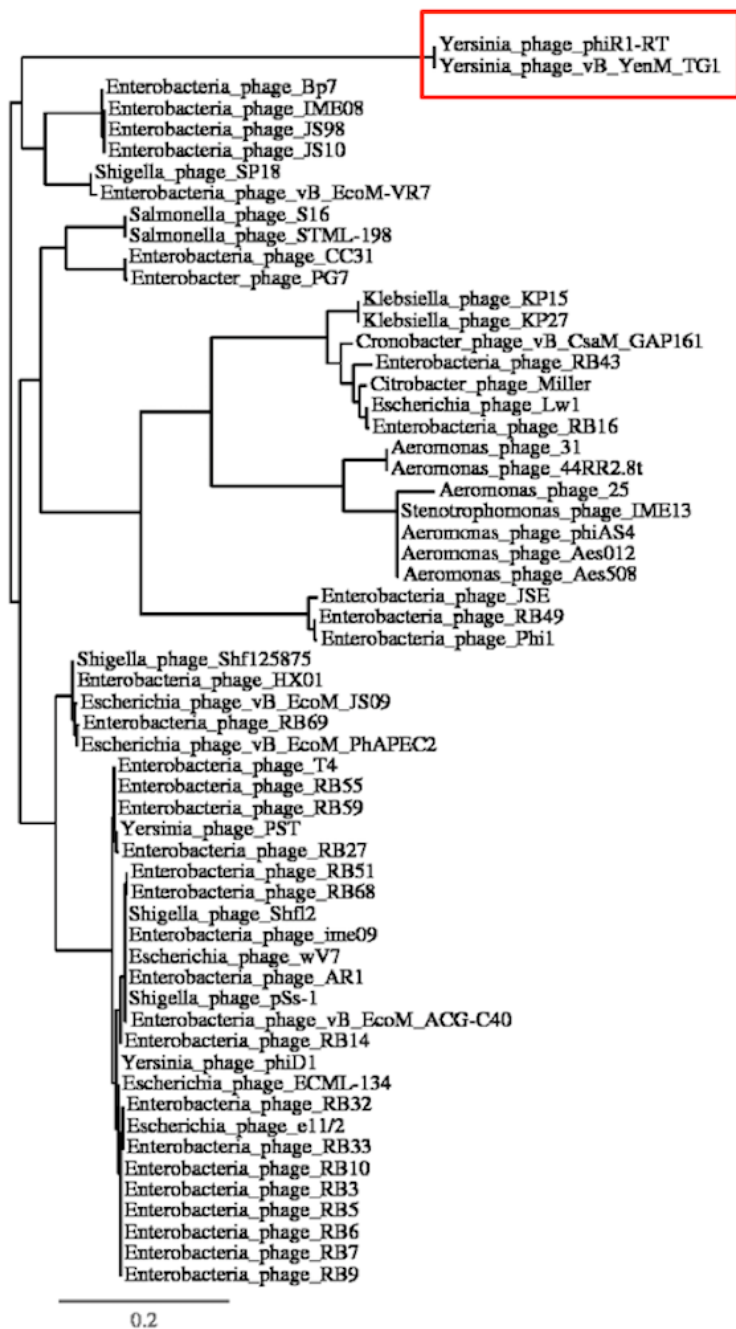


Figure 3

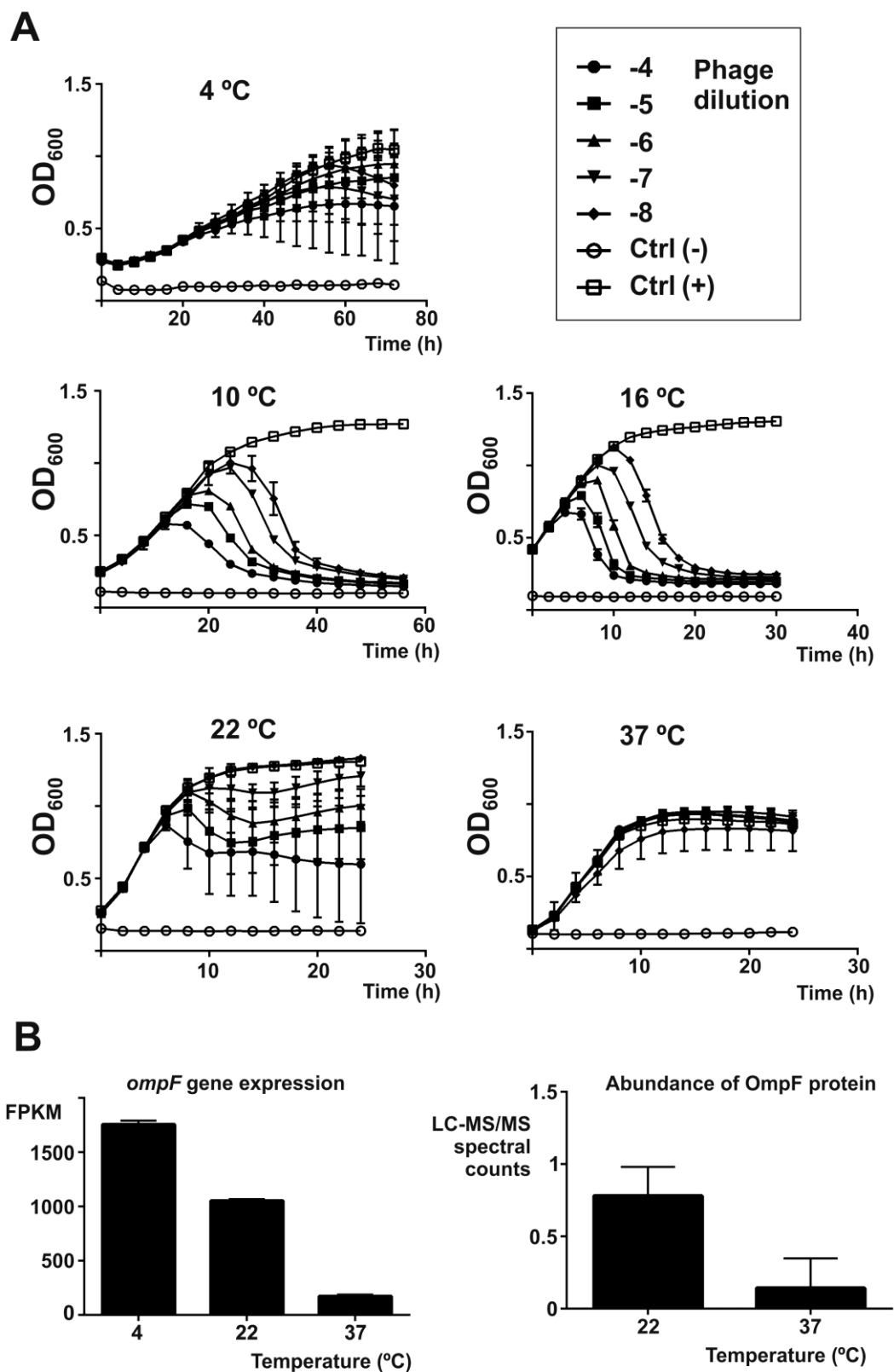


Figure 4

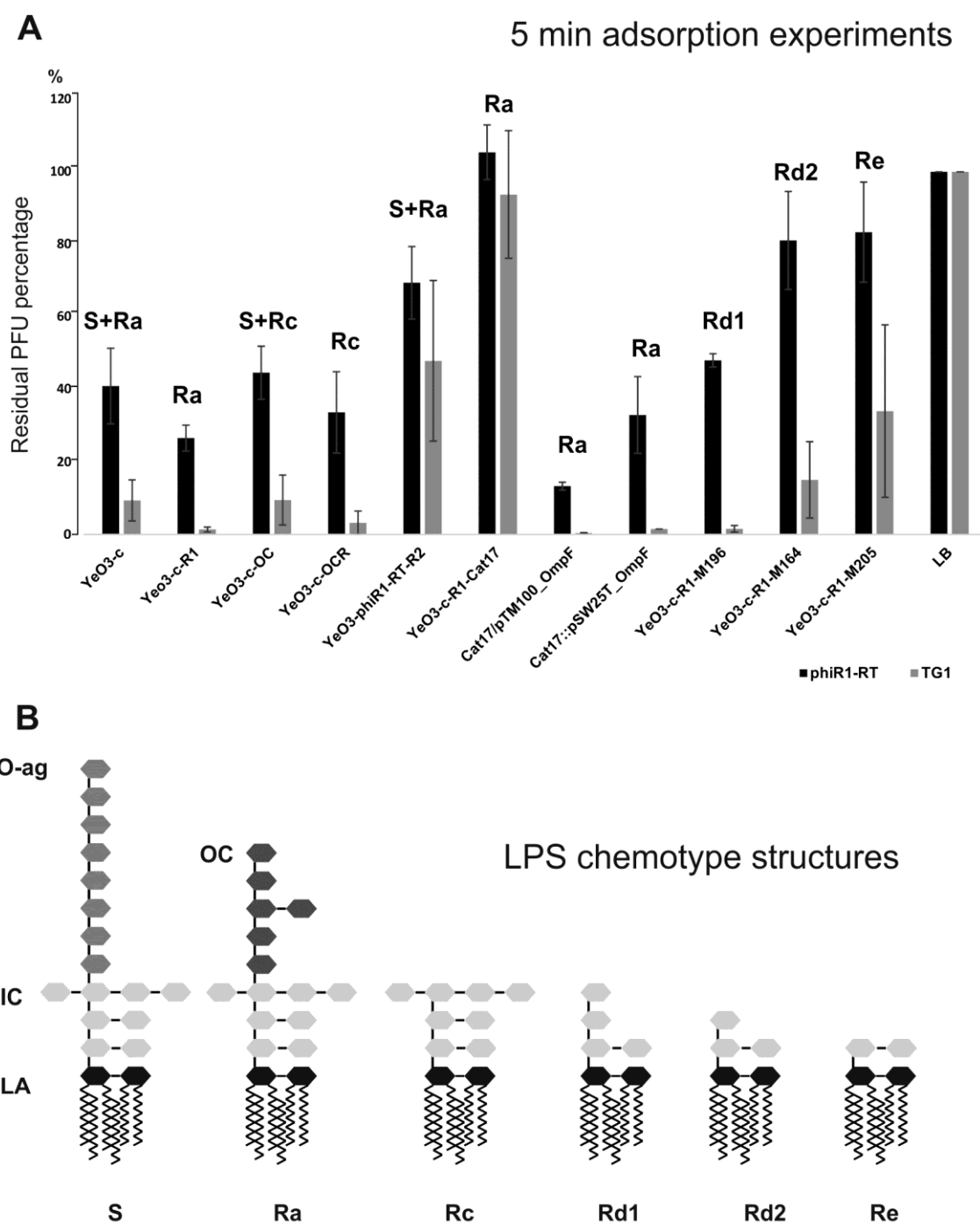


Figure 5

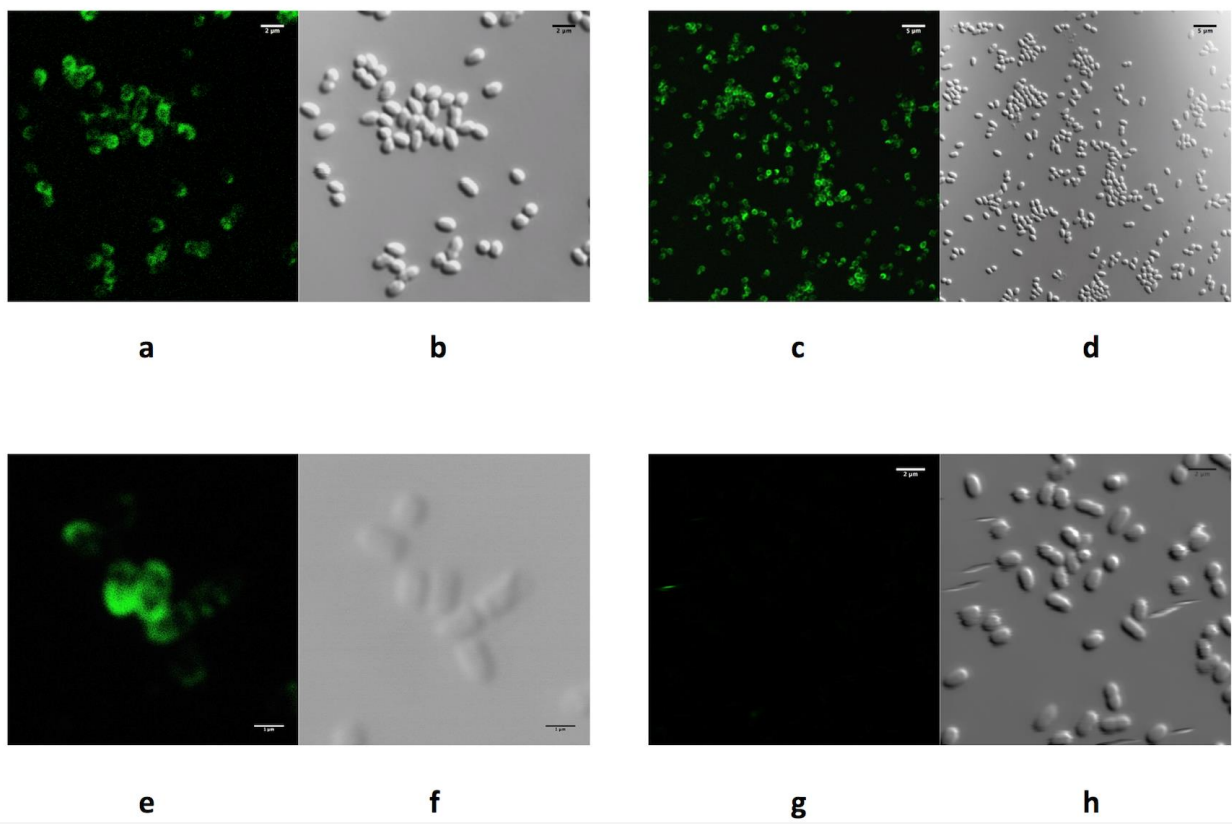


Figure 6